

# **Green Tea Polyphenols: a Natural Therapeutic Approach for Metabolic Syndrome and Diabetes Prevention**

A thesis submitted for the Degree of Doctor of Philosophy

by

Jane Jung Yeon Kim



School of Medical and Molecular Biosciences

Faculty of Science

University of Technology Sydney, Australia

2014

## **Declaration**

This thesis titled “Green Tea Polyphenols: a Natural Therapeutic Approach for Metabolic Syndrome and Diabetes Prevention” is of original work. The work presented in this thesis was carried out under the supervision of A/Prof Xianqin Qu and A/Prof Chris Zaslowski at University of Technology, Sydney. This thesis is of original work and has not been submitted by the candidate for the award of any other degree.

---

Jane Jung Yeon Kim

2014

## **Acknowledgements**

Firstly, I would like to express my gratitude to my supervisor A/Prof Xianqin Qu for her continual support, guidance, understanding and time during the years of my masters and doctoral studies. This would not have been possible without her wisdom and supervision throughout the years. Also thank you to my co-supervisor A/Prof Chris Zaslowski for his support and guidance throughout my studies too.

A big thank you to Dr Yi Tan for her endless help in the laboratory. I cannot thank her enough, for her friendship and support over the 8 years at UTS. Also, many thanks to the laboratory managers I have met along the way Phil, Ian, Mike and Harry. Many thanks for all their technical advice and assistance in the laboratory.

Thank you to my fellow PhD students and friends of level 6 who have helped me to get through the toughest times of my life.

Finally, I would like to thank my family (esp. my parents, sister Joan, husband William and son Ethan) and friends for all their love, concern and company. This thesis would not have been possible without you all.

## **Publications**

### **Publications in Peer Reviewed Journals**

**Kim, J.J.Y.,** Tan, Y., Xiao, L., Sun, Y. & Qu, X. (2013). Green Tea Polyphenol Epigallocatechin-3-Gallate Enhance Glycogen Synthesis and Inhibit Lipogenesis in Hepatocytes. *Biomed Res Int*, 2013,920128.

Lao, W.G., **Kim, J.J.Y.,** Tan, Y., Jin, X., Xiao, L. & Qu, X (2014). Green Tea Polyphenols Inhibit Adipogenesis in 3T3-L1 Preadipocytes Through Down-regulation of SREBP-1c Expression and C/EBP $\alpha$  -PPAR $\gamma$  Pathway. *J. Nutr. Biochem.* (submitted and under review).

**Kim, J.J.Y.,** Lao, W.G., Tan, Y., Xiao, L. & Qu, X. (2014). Green Tea Polyphenols Improves Insulin Resistance in High Fat Fed Obese Zucker Rats (manuscript in preparation).

**Kim, J.J.Y.,** Lao, W.G., Tan, Y., Xiao, L. & Qu, X. (2014). Effects of Green Tea Polyphenols on Non-alcoholic Fatty Liver Disease Induced by High-fat Diet in Zucker Fatty Rats (manuscript in preparation).

### **Papers and Posters Presented at Scientific Conferences**

**Kim, J.J.Y.,** Xiao, L., Tan, Y. & Qu, X. (2010). An Investigation into the Effects of Green Tea Polyphenols on Glucose Metabolism in 3T3-L1 Cells. *RNSH/UTS Scientific Research Meeting, Sydney* (Poster).

**Kim, J.J.Y.,** Xiao, L., Tan, Y. & Qu, X. (2011). The Effects of Green Tea Polyphenols on Glucose Metabolism in 3T3-L1 Cells. *Healthpac, Sydney* (Poster).

**Kim, J.J.Y.,** Xiao, L., Young, A., Tang, D.K., Sun, Y & Qu, X. (2012). Green Tea Polyphenols Improves Glucose metabolism Through Enhancing Insulin-Stimulated Glucose Uptake and Glycogen Synthesis. *Diabetes*, 61S, A682 (Poster).

## Table of Contents

Declaration.....	i
Acknowledgements.....	ii
Publications.....	iii
Table of Contents.....	v
List of Figures.....	x
List of Tables.....	xi
Abbreviations.....	xii
Abstract.....	xvii

<b>Chapter 1: Introduction .....</b>	<b>1</b>
1.1. Metabolic Syndrome.....	2
1.1.1. The Definition of Metabolic Syndrome.....	2
1.2. Components of Metabolic Syndrome .....	5
1.2.1. Insulin Resistance .....	5
1.2.2. Central Obesity .....	7
1.2.3. Glucose Intolerance .....	8
1.2.4. Dyslipidaemia .....	8
1.2.5. Hypertension.....	9
1.2.6. Pro-coagulant and Pro-inflammatory Factors.....	9
1.2.7. A Novel Component of the Metabolic Syndrome: Non-alcoholic Fatty Liver Disease .....	10
1.3. Metabolic Syndrome and Type 2 Diabetes.....	11
1.4. Metabolic Syndrome and Cardiovascular Disease .....	12
1.5. Pathogenesis of Metabolic Syndrome.....	14
1.5.1. Impaired Insulin Signalling in Skeletal Muscle.....	14
1.5.2. Adipose Tissue Dysfunction and Adipocytokines.....	17
1.5.3. Increased Ectopic Lipids and Lipotoxicity .....	19
1.5.4. Abnormalities in Hepatic Glucose and Lipid Metabolism .....	21
1.6. Current Treatment and Therapies for Metabolic Syndrome.....	25
1.6.1. Conventional Medications .....	25
1.6.1.1. Insulin-sensitising Medications .....	25
1.6.1.2. Anti-hyperlipidaemic Medications .....	26

1.6.1.3.	Anti-obesity Medications.....	27
1.6.1.4.	Anti-hypertensive Medications.....	28
1.6.2.	Lifestyle Modifications.....	28
1.6.3.	Chinese Herbal Medicines.....	29
1.6.4.	Natural Agents and Functional Foods.....	32
1.7.	Green Tea Polyphenols.....	33
1.7.1.	Active Ingredients of Green Tea Polyphenols.....	35
1.7.2.	Pharmacological Actions of Green Tea Polyphenols.....	36
1.7.3.	Anti-oxidant Effects.....	37
1.7.4.	Metabolic Effects.....	37
1.7.5.	Cardiovascular Effects.....	40
1.7.6.	Anti-cancerous Effects.....	41
1.8.	Experimental Models for Investigating New Agents for Metabolic Syndrome.....	42
1.8.1.	Cellular Models.....	42
1.8.1.1.	3T3-L1 Adipocytes.....	42
1.8.1.2.	HepG2 Cells.....	44
1.8.1.3.	L6 Cells.....	44
1.8.2.	Animal Models.....	45
1.8.2.1.	High Fat Diet Rodents.....	45
1.8.2.2.	Zucker Fatty and Zucker Diabetic Fatty Rats.....	47
1.8.2.3.	Lep <sup>ob/ob</sup> Mice.....	47
1.9.	Aims of Thesis.....	48
<b>Chapter 2: General Materials and Methods .....</b>		<b>50</b>
2.1.	Materials.....	51
2.1.1.	General Materials and Reagents.....	51
2.1.2.	Identification of Chemical Composition of GTP.....	52
2.2.	Methods for Cellular Study.....	53
2.2.1.	Cell Culture and GTP Treatment.....	53
2.2.1.1.	Maintenance and Passaging of Cell Lines.....	53
2.2.1.2.	GTP Treatment of Cells.....	54
2.2.2.	Cell Viability Assay.....	55
2.2.3.	Measurement of Glucose Uptake.....	55
2.2.4.	Measurement of Insulin-stimulated Glycogen Synthesis.....	56
2.2.5.	Lipolysis Assay.....	57

2.2.6.	Lipogenesis Assay .....	58
2.2.7.	Western Blotting .....	58
2.3.	Methods for Animal Study .....	60
2.3.1.	Experimental Animals .....	60
2.3.2.	Ethics Approval .....	60
2.3.3.	High-Fat Diet Feeding .....	60
2.3.4.	Treatment Protocol.....	60
2.3.5.	Body Weight and Food Intake .....	61
2.3.6.	Collection of Tail Vein Blood Samples .....	61
2.3.7.	Oral Glucose Tolerance Test .....	62
2.3.8.	Collection of Tissue Samples.....	62
2.3.9.	Laboratory Measurements .....	62
2.3.9.1.	Glucose Concentration.....	62
2.3.9.2.	Insulin Concentration.....	63
2.3.9.3.	Non-esterified Fatty Acids Concentration .....	63
2.3.9.4.	Triglycerides Concentration.....	64
2.3.9.5.	Cholesterol Concentration .....	64
2.3.10.	Biochemical Assays .....	65
2.3.10.1.	Western Blotting .....	65
2.3.10.2.	Determination of Alanine Transaminase and Aspartate Transaminase Concentrations .....	65
2.3.10.3.	Tissue Triglyceride Content.....	66
2.3.11.	Histological Analysis .....	67
2.3.11.1.	Specimen Preparation .....	67
2.3.11.2.	Haematoxylin and eosin Stain .....	68
2.3.11.3.	Oil red O Staining .....	68
2.4.	Statistical Analysis.....	68

### **Chapter 3: Effects and Mechanisms of Green Tea Polyphenols on Insulin-stimulated Glucose Uptake and Lipolysis in 3T3-L1 Cells .....69**

3.1.	Introduction.....	70
3.2.	Research Plan and Methods .....	72
3.3.	Results.....	74
3.3.1.	Cell viability and optimisation of GTP-EGCG concentrations using MTT assay	74



3.3.2.	Effect of GTP-EGCG on insulin-stimulated glucose uptake in 3T3-L1 cells .	76
3.3.3.	Effect of GTP-EGCG on lipolysis in 3T3-L1 cells .....	77
3.3.4.	Effects of GTP-EGCG on expressions of IRS-1, PKB/Akt and GLUT4 in 3T3-L1 cells	79
3.3.5.	Effect of GTP-EGCG on expression of PKA in 3T3-L1 cells.....	80
3.4.	Discussion .....	82
3.5.	Conclusion .....	85

#### **Chapter 4: Effects and Mechanisms of Green Tea Polyphenols on Glycogen Synthesis and Lipogenesis in HepG2 Cells.....86**

4.1.	Introduction.....	87
4.2.	Research Plan and Methods .....	90
4.3.	Results.....	92
4.3.1.	Effect of GTP-EGCG on glycogen synthesis in HepG2 Cells .....	92
4.3.2.	Effect of GTP-EGCG on lipogenesis in HepG2 cells.....	93
4.3.3.	Effects of GTP-EGCG on expressions of Ser9 pGSK3 $\beta$ and Ser641 pGS in HepG2 cells .....	96
4.3.4.	Effects on GTP-EGCG on expressions of Thr172 pAMPK $\alpha$ and Ser79 pACC in HepG2 cells .....	98
4.4.	Discussion .....	100
4.5.	Conclusion .....	101

#### **Chapter 5: Does Green Tea Polyphenols Improve Insulin Resistance in High Fat Fed Obese Zucker Rats? ..... 103**

5.1.	Introduction.....	104
5.2.	Research Plan and Methods .....	106
5.3.	Results.....	108
5.3.1.	Effect of GTP on body weight gain in HFD ZF rats.....	108
5.3.2.	Effects of GTP on serum glucose and whole body insulin resistance .....	109
5.3.3.	Effects of GTP on blood lipid profiles HFD ZF rats .....	111
5.3.4.	Effects of GTP on oral glucose tolerance in HFD ZF rats.....	113
5.3.5.	Effect of GTP on skeletal muscle in HFD ZF rats.....	114
5.3.6.	Increased GLUT4 translocation in skeletal muscle of GTP-treated HFD ZF rats	115
5.3.7.	Phosphorylation of IRS-1 and PKB/Akt in skeletal muscle of GTP-treated HFD ZF rats.....	116

5.3.8.	Translocation and expressions of PKC isoforms in skeletal muscle of GTP-treated HFD ZF rats .....	119
5.4.	Discussion .....	121
5.5.	Conclusion .....	124
 <b>Chapter 6: Effects of Green Tea Polyphenols on Non-alcoholic Fatty Liver Disease Induced By High-fat Diet in Zucker Fatty Rats.....</b>		<b>126</b>
6.1.	Introduction.....	127
6.2.	Research Plan and Methods .....	129
6.3.	Results.....	132
6.3.1.	Effects of GTP on liver enzymes ALT and AST levels in HFD ZF rats .....	132
6.3.2.	Effect of GTP on TG deposition in the liver of HFD ZF rats.....	133
6.3.3.	Effect of GTP on lipid deposition in the liver and hepatic steatosis of HFD ZF rats	134
6.3.4.	Effects of GTP on Expressions of Ser9 pGSK3 $\beta$ and Ser641 pGS in Liver of HFD ZF rats.....	136
6.3.5.	Effects of GTP on Expressions of Thr172 pAMPK $\alpha$ and Ser79 pACC in the Liver of HFD ZF rats.....	139
6.3.6.	Effect of GTP on PEPCK expression in Liver of HFD ZF rats.....	141
6.4.	Discussion .....	142
6.5.	Conclusion .....	145
 <b>Chapter 7: Final Discussion, Conclusion and Future Directions .....</b>		<b>146</b>
7.1.	Final Discussion and Conclusion.....	147
7.2.	Future Directions .....	150
 <b>References.....</b>		<b>152</b>

## List of Figures

Fig 1.1. Insulin-signalling pathway .....	15
Fig 1.2. Proposed mechanisms of green tea for T2D and obesity .....	35
Fig 1.3. Structure of green tea catechins.....	36
Fig 2.1. Structures for four polyphenols in green tea .....	53
Fig 3.1. Viability of GTP/EGCG-treated 3T3-L1 cells .....	76
Fig 3.2. Effect of GTP/EGCG on glucose uptake in 3T3-L1 cells .....	77
Fig 3.3. Effect of lipolytic activity in GTP/EGCG-treated 3T3-L1 cells .....	78
Fig 3.4. Effect of GTP-EGCG on IRS-1, PKB/Akt and GLUT4 expressions in 3T3-L1 cells.....	80
Fig 3.5. Effect of GTP-EGCG on PKA expression in 3T3-L1 cells.....	82
Fig 4.1. Glycogen synthesis in response to GTP-EGCG treatments in HepG2 cells .....	93
Fig 4.2. Effects of dose response to GTP-EGCG on lipogenesis in HepG2 cells .....	95
Fig 4.3. Effects of GTP-EGCG on expressions of phospho-GSK3 $\beta$ (Ser9) and phospho-GS (Ser641) in HepG2 cells.....	97
Fig 4.4. Effects of GTP-EGCG on expressions of phospho-AMPK $\alpha$ (Thr172) and phospho-ACC (Ser79) in HepG2 cells .....	99
Fig 5.1. Body weight gain in ZF HFD rats .....	109
Fig 5.2. Serum glucose (A) insulin (B) levels and (C) HOMA-IR in HFD ZF rats .....	110
Fig 5.3. Serum TG (A) NEFA (B) and cholesterol (C) in HFD ZF rats.....	112
Fig 5.4. Effect of GTP treatment on serum glucose during oral glucose tolerance test (OGTT) .....	114
Fig 5.6. TG content in red quadriceps.....	115
Fig 5.7. The effects of GTP on GLUT4 protein expression in HFD ZF rats.....	116
Fig 5.8. Determination of (A) IRS-1 (Ser612) and (B) AKT (Ser473) phosphorylation in skeletal muscle of GTP-treated ZF rats .....	118
Fig 5.9. Determination of PKC isoforms in red quadriceps (skeletal muscle) of GTP-treated ZF rats .....	120
Fig 6.1. Effect of GTP on alanine transaminase (ALT) (A) and aspartate transaminase (AST) (B) in HFD ZF rats.....	133
Fig 6.2. TG content in liver of HFD ZF rats .....	134
Fig 6.3. Representative haematoxylin and eosin staining of livers from (A) lean (B) HFD-Con and (C) HFD-GTP rats.....	135
Fig 6.4. Representative oil red O staining images of livers from (A) lean, (B) HFD-Con and (C) HFD-GTP rats.....	136

Fig 6.5. Determination of GSK3 $\beta$ (Ser9) and GS (Ser641) phosphorylation in the liver of ZF rats	138
Fig 6.6. Determination of AMPK $\alpha$ (Thr172) and ACC (Ser79) phosphorylation in the liver of ZF rats .....	140
Fig 6.7. Determination of PEPCK expression in the liver of ZF rats .....	141

## List of Tables

Table 1. The definition of metabolic syndrome .....	4
---	---

## Abbreviations

2DOG	2-deoxyglucose
ACC	acetyl-CoA carboxylase
AGI	Alpha-glucosidase inhibitors
AMPK	AMP-activated protein kinase
ANOVA	one-way analysis of variance
ALT	alanine transaminase
AST	aspartate transaminase
ATCC	American Type Culture Collection
ATPIII	Adult Treatment Panel III
BBR	berberine
BMI	Body mass index
BSA	bovine serum albumin
BW	body weight
cAMP	Cyclic adenosine monophosphate
CEBP $\alpha$	CCAAT-enhancer-binding protein- $\alpha$
CHM	Chinese herbal medicine
ChREBP	carbohydrate-responsive element-binding protein
CM	complete media
CREB	cAMP response element-binding protein
CRP	C-reactive protein

CVD	cardiovascular disease
DAG	diacylglycerol
DBP	diastolic blood pressure
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethyl sulfoxide
EC	epicatechin
ECG	epicatechin gallate
EGC	epigallocatechin
EGCG	epigallocatechin gallate
EGIR	European Group for the Study of Insulin Resistance
fatty acyl-CoA	fatty acyl-coenzyme A
FBS	fetal bovine serum
FFA	free fatty acids
FAS	fatty acid synthase
G-6-Pase	glucose-6-phosphatase
GDH	glutamate dehydrogenase
GLUT1	glucose transporter 1
GLUT2	glucose transporter 2
GLUT3	glucose transporter 3
GLUT4	glucose transporter 4
GOD-PAP	glucose oxidase-peroxidase
GS	glycogen synthesis

GSK3 $\beta$	glycogen synthase kinase 3 $\beta$
GTP	green tea polyphenols
HOMA-IR	homeostasis model assessment of insulin resistance
HDL	high-density lipoprotein
HE	haematoxylin and eosin
HFD	high-fat diet
FPG	fasting plasma glucose
HSL	hormone-sensitive lipase
IDF	International Diabetes Federation
IFG	impaired fasting glucose
IFN	interferon
IGT	impaired glucose tolerance
IL	interleukin
IRS	insulin receptor substrate
ISDN	isosorbide dinitrate
LC-MS	liquid chromatography-mass spectrometry
LDL	low-density lipoprotein
LKB1	liver kinase B1
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemotactic protein-1
MetS	metabolic syndrome
MI	myocardial infarction

MLC	myosin light chain
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NAD	nicotinamide adenine dinucleotide
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NCEP	National Cholesterol Education Program
NEFA	non-esterified free fatty acids
NIDDM	non-insulin dependent diabetes
NO	nitric oxide
OGTT	oral glucose tolerance test
ORO	oil red O
PAI-1	plasminogen activator inhibitor-1
PCOS	polycystic ovarian syndrome
PDE3b	phosphodiesterase 3b
PDH	pyruvate dehydrogenase
PDK1	PI-dependent kinase 1
PEPCK	Phosphoenolpyruvate carboxykinase
PI3-K	phosphoinositide 3-kinase
PKA	protein kinase A
PKB/Akt	protein kinase B
PKC	protein kinase C
PPAR $\gamma$	peroxisome proliferator activated receptor- $\gamma$



PVD	peripheral vascular disease
PVDF	polyvinylidenedifluoride
RBP4	retinol binding protein-4
SBP	systolic blood pressure
SM	starving media
SREBP-1c	sterol regulatory element binding protein-1c
STZ	streptozotocin
T2D	type 2 diabetes
TAG	triacylglycerol
TG	triglycerides
TNF- $\alpha$	tumour necrosis factor- $\alpha$
TZD	thiazolidinedione
VLDL-C	very low-density lipoprotein-cholesterol
WHO	World Health Organization
WTH	Waist-to-Hip
ZF	Zucker fatty (fa/fa)

## Abstract

Metabolic syndrome (MetS) is a collection of interrelated disorders that increase the risk of type 2 diabetes (T2D) and cardiovascular disease. Abnormalities associated with MetS include, but are not limited to, central obesity, insulin resistance, glucose intolerance, hyperglycaemia, hyperlipidaemia and hypertension. MetS has caused an encumbrance to public health globally. Due to the complex nature of MetS and the lack of availability of effective medications, there is an urgent need for the implementation of novel oral agents to manage MetS and prevent T2D and cardiovascular complications.

The beneficial effects of green tea polyphenols (GTP) for MetS have been recently reported. However, the direct effects and mechanisms of GTP on abnormalities of glucose and lipid metabolism are not fully understood. This thesis investigated effects and mechanisms of GTP in obesity, insulin resistant and metabolic dysfunctions through *in vitro* and *in vivo* studies. The effects of GTP on biochemical parameters and its actions in major organs involved in glucose homeostasis, fat metabolism and insulin sensitivity such as skeletal muscle, liver and adipose tissue were elucidated. The study was further extended to investigate the effect of GTP on a model of non-alcoholic fatty liver disease (NAFLD).

In 3T3-L1 adipocytes, GTP-EGCG (epigallocatechin gallate) improved glucose uptake and inhibited lipolysis significantly. It was revealed that GTP-EGCG significantly increased glucose uptake by up-regulating expressions of IRS-1, PKB/Akt and GLUT4. The probable mechanism for decreased lipolysis with GTP-EGCG may be through down-regulation of PKA, as observed in this thesis. GTP-EGCG also enhanced glycogen synthesis in a dose-dependent manner and significantly increased glycogen synthesis two-fold compared with insulin alone in HepG2 cells. Western blotting revealed that phosphorylation of GSK3 $\beta$  and

GS was significantly increased in GTP-EGCG treated HepG2 cells compared to non-treated cells. GTP-EGCG also significantly inhibited lipogenesis and the likely mechanism behind this inhibition involved enhanced expression of phosphorylated AMPK $\alpha$  and ACC in HepG2 cells.

A marked state of insulin resistance was observed in high fat diet (HFD) fed obese Zucker fatty (ZF) rats compared to their lean littermates, which is associated with significant defects in the insulin-signalling/glucose transport system. GTP significantly improved fasting metabolic parameters, including glucose, insulin, TG, NEFA and cholesterol, and improved insulin sensitivity and glucose intolerance in HFD ZF rats. The likely molecular mechanisms involved are regulation of the elements of the insulin-signalling pathway such as PKB/Akt, GLUT4 translocation and regulation of PKC translocation in skeletal muscle of HFD ZF rats. Data from this thesis also strongly supports that GTP reduces fat accumulation in the liver and hepatic insulin resistance. This is evidenced by significant reduction in serum levels of ALT and AST hepatic TG and lipid content in HFD ZF rats administered GTP. Mechanisms are likely to up regulate GSK3 $\beta$  and GS expressions thereby enhancing glycogen synthesis and down-regulating *de novo* lipogenesis through regulation of AMPK $\alpha$ , ACC and PEPCK expressions.

Overall, the results presented in this thesis provide insight into GTP as a potential therapeutic agent for MetS and related disorders such as T2D, obesity and NAFLD. GTP may be a valuable natural and cost-effective therapy for the treatment and prevention of metabolic disorders.